Claims

1. A method for the isolation of an scFv with defined framework that is stable and soluble in a reducing environment, wherein

a) a scFv library with varied frameworks and constant CDRs is generated by mutation of at least one framework encoding region of DNA sequence of a scFv to a known antigen and by introduction of such mutations into suitable expression vectors,

b) host cells able to express a specific known antigen and only surviving in the presence of antigen-scFv-interaction are transformed with said scFv library,

c) the thus transformed host cells are cultivated under conditions suitable to express the antigen and the scFv and allowing cell survival only in the presence of antigen-scFv-interaction,

d) the scFv expressed in surviving cells and having a defined framework that is stable and soluble in reducing environment is isolated.

2.The method of claim 1, wherein the host cell is an eukaryotic cell.

3. The method of claim 2 wherein the host cell is a yeast cell.

4. A scFv with defined framework, obtainable by the method of claim 1.

5. The scFv of claim 4 comprising restriction sites allowing the selective exchanging of at least one CDR.

6.The scFv of claim 5, wherein the restriction sites are located within the framework flanking a CDR.

7. A method for the generation of a scFv encoding DNA with a framework suitable for selective alterations in the CDR region, wherein specific restriction sites are introduced into the sequence of a defined, sta-

ble and soluble scFv encoding DNA by means of site directed mutagenesis.

8. The method of claim 7, wherein the restriction sites are located within the framework and whereby the substitution of the nucleotides to generate the restriction site does not affect the amino acid sequence.

9. A method for the generation of a scFv with defined framework that is stable and soluble in a reducing environment, wherein at least two variations of at least two different frameworks isolated according to claim 1 that are stable and soluble in a reducing environment are combined to produce a scFv with defined framework.

10. A scFv with defined framework, obtainable by the method of claim 9.

11. The scFv of claim 10 wherein the variations are preceding the CDR1 of the variable light chain.

12. The scFv of claim 10 wherein the variations are located between CDR2 and CDR3 of the variable heavy chain.

13. The scFv of claim 10 wherein at least one variation is preceding the CDR1 and at least one variation is located between CDR2 and CDR3 of the variable heavy chain.

14. The scFv of claim 10 wherein at least 2 variations are preceding CDR1 and at least 2 variations are located between CDR2 and CDR3 of the variable heavy chain.

15. A scFv comprising the framework defined in SEQ ID NO 1.

16. A method for the generation of a CDR library with a defined framework, that is stable and soluble in a reducing environment, wherein DNA sequences encoding a scFv of one of the previous claims are digested to replace at least one CDR per sequence by a modified CDR.

17. The method of claim 16, wherein the modified CDR is generated by random changes.

18. A library of intrabodies with at least one randomized CDR and defined framework that is stable and soluble under reductive conditions.

19. A method for screening for CDRs interacting with a specific antigen, wherein host cells transformed with a nucleic acid sequence encoding a known antigen are further transformed with a randomized CDR library with defined framework that is stable and soluble in a reducing environment, whereby the antigen and/or the scFv are linked to a marker system or part of a marker system thus that the cell cultured under selective conditions only survives in the presence of antigen/scFv-interaction, that thus transformed cells are cultivated under selective conditions, and that surviving cells are cultured and the intrabodies harvested.

20. The method of claim 19, wherein the framework is a framework as defined in one of the preceding claims.

21. The method of claim 19, wherein the cell is an eukaryotic cell.

22. The method of claim 19 wherein the DNA sequence encoding the antigen and the DNA sequence encoding the scFv both encode chimeric molecules with the antigen or scFv, respectively, both linked to part of a transcription activating system linked to a survival allowing marker.

23. The method of claim 22, wherein the antigen is fused to a DNA binding domain and the scFv is fused to a transcriptional activator domain or the antigen is fused to a transcriptional activator domain and the scFv is fused to a DNA binding domain.

24. A method for screening for an antigen interacting with an scFv, wherein host cells expressing at least one antigen of interest are transformed with at least one scFv with defined framework that is stable and

soluble in reducing environment, or with a randomized CDR library with defined framework that is stable and soluble in reducing environment, whereby the antigens and/or the scFvs are linked to a marker system or part of a marker system thus that the cell cultured under selective conditions only survives in the presence of antigen/scFv-interaction, that thus transformed cells are cultivated under selective conditions, and that surviving cells are cultured and the scFvs harvested.

25. The method of claim 24, wherein the framework is a framework as defined in one of the preceding claims.

26. The method of claim 24, wherein the cell is an eukaryotic cell, in particular a yeast cell.

27. The method of claim 24, wherein the DNA sequence encoding the antigen and the DNA sequence encoding the scFv both encode chimeric molecules with the antigen or scFv, respectively, both linked to part of a transcription activating system linked to a survival allowing marker.

28. The method of claim 27, wherein the antigen is fused to a DNA binding domain and the scFv is fused to a transcriptional activator domain or the antigen is fused to a transcriptional activator domain and the scFv is fused to a DNA binding domain.

29. An scFv with defined framework as therapeutic or diagnostic or prophylactic agent.

30. Use of the scFv with defined framework for intracellular screenings.

31. A method for the identification of intrabody frameworks or intrabodies wherein suitable host cells are transformed with a library and a marker system, whereby said library is a fusion product of an intrabody library and at least part of said marker system, wherein said marker system is only activated in the presence of a fusion protein encoding a soluble and stable intrabody framework, and culturing said cells under conditions al-

lowing the identification and selection of cells expressing a soluble and stable intrabody framework.

32. The method of claim 31, wherein said library is a fusion product of an intrabody library and a marker protein.

33. The method of claim 32, wherein said marker protein has a selectable activity.

34. The method of claim 33, wherein said selectable activity is an enzymatic activity or a fluorescene activity.

35. The method of claim 31, wherein said library is a fusion product of an intrabody library and a DNA binding protein that can activate transcription.

36. The method of claim 31, wherein said suitable host cells are transformed with a library that encodes proteins comprising an intrabody and one part of a transactivation system and said cells further express a second protein comprising the second part of said transactivation system, whereby said transactivation system is linked to a survival allowing marker and said cells only survive under selective conditions in the presence of an interaction between said two proteins.

37. The method of claim 36, wherein said library encoded proteins comprise a transcriptional activation domain and said second proteins comprise a DNA binding domain or said library encoded proteins comprise a DNA binding domain and said second proteins comprise a transcriptional activation domain.

38. The method of claim 37, wherein said second proteins comprise a DNA binding domain or a transactivation domain, respectively, and a protein interacting with a constant region of said first library encoded protein.

39. A scFv with defined framework obtainable by the method of claim 31.

40. The method of claim 19, wherein the nucleic acid sequence is a DNA sequence.

41. The method of claim 21 wherein the eukaryotic cell is a yeast cell.